

Cardiac metabolism during exercise in healthy volunteers measured by ^{31}P magnetic resonance spectroscopy

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Abstract

A technique was devised for individuals to exercise prone in a magnet during magnetic resonance spectroscopy of the heart and phosphorus-31 magnetic resonance spectra of the heart were obtained by the phase modulated rotating frame imaging technique in six healthy volunteers during steady state dynamic quadriceps exercise. During prone exercise heart rate, blood pressure, and total body oxygen consumption were measured at increasing loads and the results were compared with those during Bruce protocol treadmill exercise. During prone exercise with a 5 kg load the heart rate was similar and the systolic and diastolic blood pressures were higher than those during stage 1 of the Bruce protocol. The rate-pressure products were similar but the total body oxygen consumption was lower during prone exercise. There was no difference in the ratio of phosphocreatine to adenosine triphosphate during rest and exercise.

Thus during exercise that produced a local cardiac stress equal to or greater than that during stage 1 of the Bruce protocol treadmill exercise, the energy requirements of the normal human myocardium were adequately supplied by oxidative phosphorylation.

The relation between energy provision and mechanical function of the myocardium in healthy and diseased states is fundamental to cardiology. Over the past decade, phosphorus-31 magnetic resonance spectroscopy has been shown to be a powerful tool for the study of tissue energetics in isolated perfused rodent hearts.¹ The technique has also been applied in surgically exposed animal hearts.²⁻⁴ The relations between the concentrations of high energy phosphate compounds such as phosphocreatine and adenosine triphosphate and intracellular pH and mechanical function during ischaemia and reperfusion as well as the effects of abnormal endocrine function such as hyperthyroidism have been extensively studied.^{5,6}

More recently, the development of large bore magnets has enabled phosphorus spectra of the human heart to be obtained non-invasively in healthy volunteers at rest.⁷⁻⁹ The

addition of proton decoupling improved the resolution of the phosphorus spectra.¹⁰ Some success has been achieved in obtaining spectra of hearts of patients with hypertrophic cardiomyopathies and myocardial infarction at rest.^{11,12} No striking abnormalities were seen in the concentrations of high energy phosphates in the hearts of patients with cardiomyopathies.¹¹ This accords with the observation that other methods of cardiac assessment do not show abnormalities of function when the patient is studied at rest. Many indices of cardiac function are abnormal when the patient is stressed. To obtain phosphorus-31 magnetic resonance spectra of the human heart during exercise, an exercise stress test for use in whole body magnets is required. Exercising in the confined space of a magnet is difficult. The subject is required to lie face down (to reduce the distance between the coil and the myocardium) with the heart in the middle of the homogeneous magnetic field of a 60 cm bore tube. Movement of the thorax must be minimal to ensure accurate localisation. Because it takes 15-30 minutes to obtain a phosphorus-31 spectroscopic image of the heart, the patient must be able to maintain a steady level of exercise for this period.

To overcome these difficulties we developed a new exercise machine and protocol of prone exercise (pronex) in which the subjects lie face down in the bore of the magnet with the chest immobilised over the spectroscopy probe and exercise by extending their foreleg. We measured heart rate, blood pressure, rate-pressure products, and total body oxygen consumption at different workloads and compared these results with the measurements in the same individuals during the standard Bruce protocol treadmill test. We then obtained phosphorus-31 spectra from the hearts of five individuals at rest and during one level of prone exercise. An outline of the results was reported in a letter to the *Lancet*.¹³ We compared the results of physiological measurements during stage 2 of prone exercise and stage 1 of the Bruce protocol in six healthy volunteers and measured the ratio of phosphocreatine to adenosine triphosphate by magnetic resonance in five.¹³

In the present paper we report the physiological response at different levels of exercise and the spatially resolved spectra in the same six healthy volunteers. We also studied seven more individuals to determine the reproducibility and steady state response during prone exercise.

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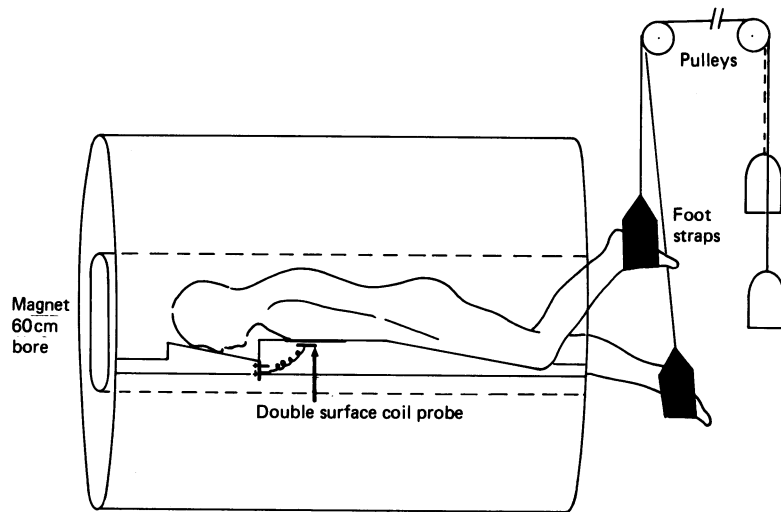


Figure 1 Arrangement for prone exercise in the bore of a horizontal magnet. The heart lies in the middle of the homogeneous field. Each foot is suspended in a strap connected to a 5 kg weight via a cable.

Subjects and methods

The studies were performed in two stages. Preliminary assessments were made in the exercise laboratory on a specially constructed rig, with a similar design to the machine that was attached to the magnet (fig 1). The rig consisted of an A frame (180 cm by 100 cm by 80 cm) to which pulleys were attached and a modified examination couch. Cylinders were used for weights and the load was increased by adding water from reservoirs attached to the apex of the A frame. The subject lay prone on the couch with each foot in a separate strap suspended on a cable. This position corresponded to that in the bore of the magnet and provided ideal stability of the thorax.

EXPERIMENTAL PROTOCOL

Individuals were examined and weighed. Then they lay on a mat and the approximate weight of each foreleg was measured by small scales. The subject then climbed on to the couch and was connected to the machine. They gripped a Lloyd respiratory valve in their mouth; a nose clip was applied; and a three minute sample of resting expired air was collected in a Douglas bag. The subject rested for five minutes and the heart rate and blood pressure were measured from a monitor (Cardiac Recorders) and manual sphygmomanometer. To record blood pressure the left arm was abducted and supported on a frame at an angle of approximately 45° from the horizontal. After collection of the resting expire exercise started. Individuals moved their forelegs in time to a metronome set at 100 per minute such that each leg moved fifty times per minute. The weights were raised approximately 25 cm with each leg extension. The subjects were asked to hold the weights suspended just above the floor at the end of each relaxation. Expired air was collected during the third, eleventh, and nineteenth minutes of exercise. Subjects lifted 2.5 kg during the first three minutes, which was equivalent to the estimated weight of the right

forelimb in most individuals. This was increased by 1.25 kg during the fourth, eighth, twelfth, and sixteenth minutes of exercise such that during collection of the second and third samples of expired air the workloads were 5 kg and 7.5 kg respectively. The samples of expire were kept sealed in the Douglas bags until the end of the experiment. A 30 ml sample of the expired air was then drawn into a glass syringe and the volume of air in the bags was measured with a dry gas meter (Parkinson Cowan). These samples were then analysed for oxygen, nitrogen, and carbon dioxide by a mass spectrometer (Centronics). Oxygen consumption was calculated from the Haldane equation with adjustments for the prevailing barometric pressure, temperature, and nitrogen and oxygen concentrations in room air.

The same six individuals also performed treadmill exercise according to the Bruce protocol on a separate day. Blood pressure and heart rate were recorded every minute and the total body oxygen consumption was measured by the above procedure from samples of expired air collected during the third minute of each of stages 1 to 4. The steady state response and reproducibility of the changes during prone exercise were assessed in four and five of the seven extra subjects respectively.

MAGNETIC RESONANCE SPECTROSCOPY

Magnetic resonance spectroscopy was performed with a 1.9 T, 60 cm bore superconducting magnet (Oxford Instruments) interfaced with a Fourier transform spectrometer (Bruker) operating at frequencies of 32.701 and 80.783 MHz for ^{31}P and ^1H respectively. The phase modulated rotating frame imaging technique that we used for spectroscopy has been described elsewhere.¹⁴ Recordings were made from the subject lying prone with the heart positioned over a double concentric surface coil (transmitter diameter 15 cm and receiver diameter 6.5 cm). We used the proton signal to optimise the magnetic field homogeneity ("shimming"). Phosphorus-31 spectroscopic images were collected from the anterior aspects of the right and left ventricles and adjacent septum by acquiring two datasets, each consisting of 16 incremental pulse widths of 16 scans at a repetition rate of 3 seconds (total time per image = 28 minutes). We used pulse sequence:

$$n\Delta\theta \pm x - \lambda y - Aq - \text{Delay}$$

where n is the number of the increment, $\Delta\theta$ the length of the pulse, λ the phase encoding pulse (optimally $\pi/2$ at sample centre), and Aq , the acquisition time (0.512 s). In our experiments (using 140 W radiofrequency transmitter power) both $\Delta\theta$ and λ were set at 350 μs . The distance was calibrated from phantom experiments and by this protocol the spatial resolution at half height of the technique was estimated to be 5–6 mm. Spectra from the heart were identified from the plateau in the ratio of phosphocreatine to adenosine triphosphate as described else-

Table 1 Data on main subject population at rest and upright

No	Age (yr)	Wt (kg)	Heart rate (beats/min)	SBP/DBP (mm Hg)	$\dot{V}O_2$ (ml/kg/min)
1	26	73	84	112/68	3.7
2	25	65	53	110/74	4.1
3	24	66	58	122/76	4.2
4	25	64	45	110/70	4.0
5	24	71	52	120/74	4.0
6	28	73	74	108/74	3.0

Mean variables among other subjects also used for steady state and reproducibility studies (mean (SD))

n	Age	Wt (kg)	Heart rate (beats/min)	SBP	DBP	$\dot{V}O_2$
7	40 (8)	74 (5)	72 (6)	123 (4)	75 (9)	3.6 (0.3)

SBP, systolic blood pressure; DBP, diastolic blood pressure; $\dot{V}O_2$, total body oxygen consumption.

where.¹⁴ The peaks in the spectra were measured by triangulation of the areas under the phosphocreatine and γ adenosine triphosphate peaks. We expressed the results as ratios because the measurement of absolute concentrations of metabolites by surface coils is not yet possible.¹⁵ In any case, the ratio of phosphocreatine to adenosine triphosphate adequately expresses the energy state of heart muscle.¹⁶ The measurement of myocardial pH in vivo requires the identification of the signal from intracellular inorganic phosphate. Unfortunately, in vivo the peaks of 2–3 diphosphoglycerate from red blood cells and extracellular inorganic phosphate overlap with the intracellular inorganic phosphate, so that with current techniques pH cannot be measured.^{17,18} Because a value for pH is necessary for the calculation of free adenosine diphosphate from the creatine kinase equilibrium, we could not calculate free adenosine diphosphate values.

Subjects exercised in the magnet using the arrangement shown in fig 1. The subject lay prone on the probe with the forelegs protruding from the bore of the magnet. Each foot was attached to a strap and a stainless steel cable. The latter was connected via pulleys (suspended on aluminium uprights bolted to the magnet) to brass weights that were attached to the wall of the magnet room. The end of the magnet was enclosed in a Faraday cage to avoid the introduction of

noise into the spectrometer by external sources of radiofrequency.

Once the resting spectra had been collected the subject exercised for two minutes while the shimming was rechecked. (The subjects were not removed from the magnet between the rest and exercise recordings.) Spectra were then collected throughout the remaining 28 minutes of exercise. The workload was 5 kg and the rate of pulling was 50 per minute per leg. Heart rate and cardiac rhythm were monitored electrocardiographically by electrodes attached to the abdominal wall.

SUBJECTS

The main study was performed on six men who were drawn from the 13 subjects who participated in the physiological characterisation and assessment of the reproducibility of prone exercise. All were healthy and had no symptoms or signs of cardiac disease. Table 1 shows the cardiorespiratory variables in upright resting subjects. All subjects consented to the study which was approved by the local ethics committee. Statistical analysis was performed by analysis of variance and Student's *t* test.

Results

The results of the measurements performed in the five main parts of the study are shown below—that is, the physiological changes during incremental prone exercise, during Bruce protocol treadmill exercise, during steady state prone exercise, and during the reproducibility studies. The spectra from the myocardium at rest were then compared with those during prone exercise in five men.

PHYSIOLOGICAL CHANGES DURING PRONE AND TREADMILL EXERCISE

Table 2 shows heart rate, blood pressure, and total body oxygen consumption in six men lying prone at rest and during two exercise protocols. Systolic and diastolic blood pressures (mm Hg) were higher in the prone position at rest than in the upright position (114 (6)/73 (3) *v* 124 (8)/84 (8); *p* < 0.02). The resting heart rate was similar in both the upright and prone positions. The mean value for the heart rate at stage 3 of the prone exercise was intermediate between stage 2 and stage 3 of the Bruce protocol. The mean systolic blood pressures at each stage were only slightly higher during treadmill exercise. There was a considerable difference in the response of the diastolic blood pressure. This fell during treadmill exercise whereas the higher resting dias-

Table 2 Physiological changes (mean (SD)) during incremental prone exercise and treadmill exercise in six individuals

	Heart rate (beats/min)	SBP (mm Hg)	DBP (mm Hg)	$\dot{V}O_2$ (ml/kg/min)
<i>Pronex</i>				
Rest	58 (10)	124 (8)	84 (8)	3.6 (0.4)
Stage 1	73 (20)	129 (8)	92 (5)	6.0 (0.2)
Stage 2	86 (19)	146 (16)	100 (6)	9.2 (2.0)
Stage 3	119 (30)	172 (22)	116 (11)	13.3 (2.1)
<i>Treadmill</i>				
Rest	58 (15)	114 (6)	73 (3)	3.8 (0.4)
Stage 1	91 (15)	134 (17)	73 (6)	14.5 (2.9)
Stage 2	103 (16)	147 (20)	70 (6)	18.9 (2.8)
Stage 3	137 (15)	158 (26)	68 (6)	26.7 (4.1)
Stage 4	168 (20)	162 (30)	67 (13)	35.1 (5.4)

See footnote to table 1 for abbreviations.

Table 3 Rate-pressure product (mean (SD)) during pronex and treadmill exercise in six healthy individuals

	<i>Pronex</i>	<i>Treadmill</i>
Rest	7267 (1389)	6916 (1606)
Stage 1	9461 (2852)	12290 (3028)*
Stage 2	12670 (3580)*	15810 (4088)
Stage 3	20569 (6992)	22287 (5273)
Stage 4		22459 (6880)

*NS.

Table 4 Steady state prone exercise (mean (SD)) results in four of seven healthy individuals

Variable	Rest	3 min	11 min	19 min
HR	76 (12)	90 (13)*	91 (15)*	91 (13)*
SBP	126 (6)	145 (3)*	140 (5)*	144 (4)*
DBP	81 (6)	92 (3)*	93 (4)*	92 (3)*
Vo ₂	3.4 (0.3)	7.8 (0.9)*	8.8 (1.8)*	8.4 (1.2)*

*None of the variables changed significantly during the prone test.

HR, heart rate. See footnote to table 1 for other abbreviations.

tolic blood pressure rose as the workload was increased during prone exercise. The difference in the diastolic blood pressure between both types of exercise was highly significant ($p < 0.0001$).

The oxygen consumption was equivalent at rest before both types of exercise and was similar to the normal values reported by other investigators.¹⁹ During treadmill exercise the oxygen consumption was slightly lower at each stage than that reported elsewhere. The oxygen consumption at stage 3 of the prone exercise was similar to that at stage 1 of the Bruce protocol.

Table 3 shows the rate-pressure products of the two types of exercise. They show that high

Table 5 Reproducibility of the prone exercise protocol (mean (SD)) in five of seven healthy individuals

Variable	Rest 1	Rest 2	Exercise 1	Exercise 2
HR	65 (12)	63 (13)	86 (21)	82 (19)
SBP	120 (9)	121 (7)	132 (14)	133 (14)
DBP	83 (8)	84 (8)	89 (8)	93 (5)
Vo ₂	3.38 (0.16)	3.43 (0.22)	7.95 (1.66)	7.4 (1.2)

See footnote to table 1 for abbreviations.

rate-pressure products can be achieved during prone exercise. Indeed during the final stage of the prone exercise protocol the rate-pressure product was over 20 000 and was close to those during stages 3 and 4 of the Bruce protocol.

PHYSIOLOGICAL CHANGES DURING STEADY STATE PRONE EXERCISE

The changes in the oxygen consumption and haemodynamic variables in four subjects exercising at constant workload (5 kg) for 20 minutes were studied to determine the variability of the response to prone exercise (table 4). Whole body metabolism was in a reasonably steady state throughout the exercise (3 min *v* 11 min *v* 19 min) indicating that the magnetic resonance spectra were obtained during steady state cardiac metabolism.

REPRODUCIBILITY OF PHYSIOLOGICAL MEASUREMENTS

Reproducibility of the measurements was examined by measuring the haemodynamic variables at rest and during exercise on two separate days within a six week period (table 5).

MAGNETIC RESONANCE SPECTROSCOPY

Spectra from the myocardium were successfully recorded in five of the six subjects. The signal to noise in the spectra from the sixth subject was not adequate for analysis because of the large distance between the myocardium and the probe. Figure 2 shows typical spectra at rest from one of the subjects. (These are comparable to the spectra acquired from this subject 3 years ago.)¹⁴ The ratio of the phosphocreatine to adenosine triphosphate at rest was similar to that in the spectra recorded in the subject during 28 minutes of steady state exercise. The mean ratio of phosphocreatine to adenosine triphosphate in the five subjects at rest (1.5 (0.2); mean (SD)) was not significantly different from that during exercise (1.58 (0.14)). The workload (5 kg) used during stage 2 of the incremental prone exercise was used for the exercise during spectroscopy. This level of work was chosen because it could be sustained during the period required for data acquisition. It caused an approximate threefold increase in total body oxygen consumption and a rate-pressure product that was similar to that at stage 1 of the Bruce protocol.

Discussion

This study was designed to evaluate a new technique of exercise for study of the human heart and to describe the pattern of phosphorus-31 magnetic resonance spectra during light exercise in healthy volunteers.

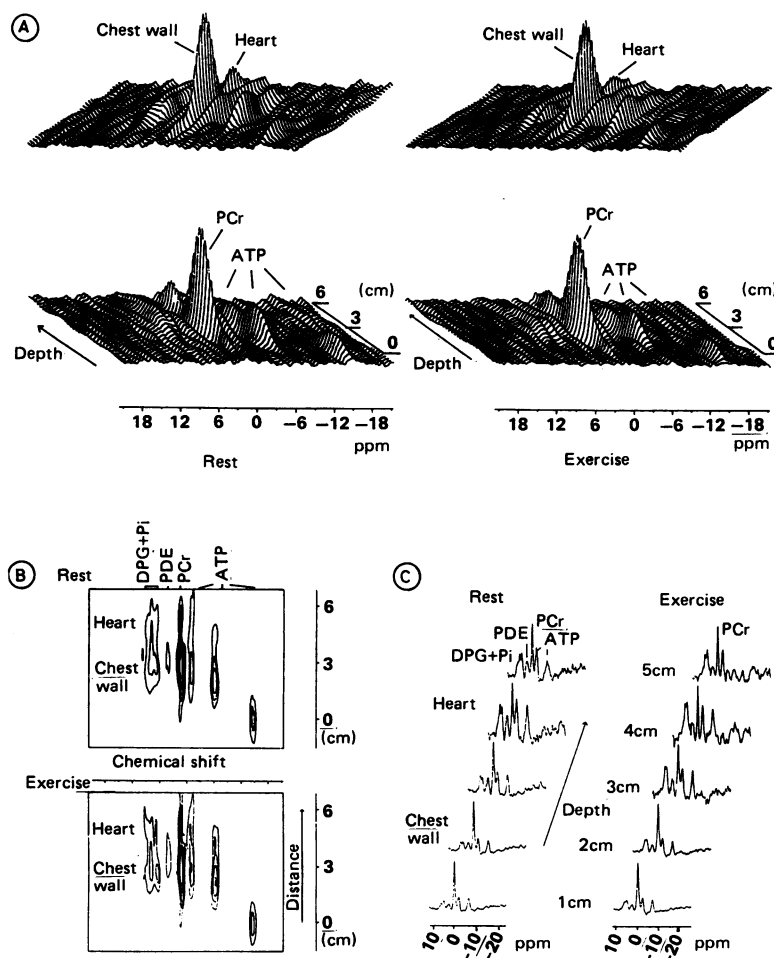


Figure 2 (A) Double projection stack plots of phase modulated images of the chest wall and heart at rest and during exercise obtained by the rotating frame technique. The heart is clearly seen in the deeper regions of the image. (B) The corresponding contour plots of the rest and exercise study which allow identification of the heart in the deeper regions. (C) The spectra were selected from corresponding rows in each image. Pi, inorganic phosphate; DPG, diphosphoglycerate; PCr, phosphocreatine; ATP, adenosine triphosphate; PDE, phosphodiester.

The constraints of the spectroscopy technique require the development of methods of exercise suitable for studies of individuals lying prone and in a fixed position that are applicable in patients with myocardial disease. Several methods of exercise and other stress techniques could have been attempted. A modification of the arm crank exercise²⁰ is possible but is less suitable because of the smaller muscle bulk and movement of the upper thorax. Supine bicycle ergometry is also unsatisfactory because in this position the myocardium is furthest away from the surface coil. The need for a long recording time precludes magnetic resonance spectroscopy after exercise outside the magnet, and sustained isometric exercise over long periods is exhausting.²¹ Though pacing²² and pharmacological stimulation²³⁻²⁵ could be used to solve the technical problems associated with movement they are at best a close approximation to the physiological response to exercise.

Prone dynamic exercise of the quadriceps muscles solves most of the problems associated with exercise in horizontal magnets and the haemodynamic changes elicited meant that this was a good method of applying a local cardiac stress with low level muscular exercise. This type of exercise does not seem to have been studied before. Treadmill and upright bicycle ergometry have been evaluated extensively^{19,26} and several investigators have examined the physiological changes during supine bicycle ergometry.²⁷ In contrast with the findings in these types of exercise there was an unusually large rise in the diastolic blood pressure in all the subjects during prone exercise. The rate-pressure product is similar to that during the treadmill test. In contrast, total body oxygen consumption is lower in prone exercise. Because for each individual the rate-pressure product is almost linearly related to myocardial oxygen consumption,²⁸ these results suggest that the same myocardial stress can be achieved at a lower total body oxygen consumption during our prone exercise protocol.

A diastolic blood pressure of >120 mm Hg was recorded in several subjects during the final stage of prone incremental exercise. This pressure is similar to those reported in some subjects during isometric exercise.²¹ The isometric exercise is probably performed by the hamstrings because the subject is required to hold the weight suspended in the weight carrier between each elevation. The back muscles also perform isometric exercise in stabilising the pelvis. A further factor that may be relevant to the increase in cardiac work is the change in blood volume distribution. Cardiac volume is greater during horizontal supine exercise²⁹ and though lying on the chest may affect intra-thoracic pressures, some increase in cardiac volume is likely. The workloads that we used in the present protocol were derived from the weight of the forelegs. This was initially determined for each leg by filling the cylinder until its weight exactly balanced that of the prone foreleg and caused the leg to flex. The weight was similarly estimated by resting the prone foreleg (at the instep) on small scales. Most subjects registered 2.5 kg for the right leg and

so this was used as the initial workload. All the subjects were able to perform exercise at the 5 kg workload for the required length of time without interruption.

Magnetic resonance spectroscopy was performed without cardiac or respiratory gating. Thus the measurements are the mean of systolic and diastolic. Comparison of systolic and diastolic measurements of phosphorus metabolites in beating animal hearts³⁰ did not show any differences between the phases of the cardiac cycle; and spectra collected from human hearts by the phase modulated rotating frame imaging technique in parallel studies were not altered by gating. The sensitive volume of the probe used in these studies is a cylinder coaxial with the probe whose diameter is the same as that of the receiver coil (6.5 cm) and increases by about 25% at a depth of 5–6 cm. With this configuration and the position of the probe as determined by echocardiograms, only the heart is seen by the probe. Moreover, if the diaphragm had been included in the sampled volume, because skeletal muscle has a high ratio of phosphocreatine to adenosine triphosphate, the images would have shown higher ratios than were detected.

Measurements of the ratio of phosphocreatine to adenosine triphosphate are used as a measure of energy metabolism. Absolute values for these metabolites cannot be derived accurately with current technology. This is because the sensitivity of the surface coils has a complex shape and varies in space depending on various factors such as electrical loading by the tissues.¹⁵ These factors have not yet been elucidated in sufficient detail for absolute concentrations to be estimated. The presence of a large peak for the 2,3 diphosphoglycerate in blood at the frequency of the inorganic phosphate interferes with the measurement of pH.

The ratio of phosphocreatine to adenosine triphosphate was no different during rest and exercise. The absence of change in this ratio during exercise suggests that the energy requirements of the heart are adequately supplied by oxidative phosphorylation during light exercise. This indicates that during ordinary increases in exertion such as walking or light running myocardial metabolism is solely oxidative and phosphocreatine is not broken down. In the skeletal muscle of healthy individuals there was little change in phosphocreatine and pH fell during light exercise.³¹

The metabolism of the diseased myocardium during exercise has not been studied yet. It is likely that there are considerable changes in phosphocreatine resembling the abnormalities in the skeletal muscle of patients with heart failure.³¹ Prone exercise should be an efficient method of studying the ischaemic myocardium because a large local haemodynamic stress can be produced by relatively low muscular effort. The applicability of the exercise technique in middle aged patients has already been examined. The level of exercise can be tailored to each individual and significant changes in haemodynamic variables would be expected with workloads as low as 2.0–2.5 kg. Exercise magnetic resonance spectroscopy studies will

require adequate cardiac monitoring with the electrocardiograph to detect arrhythmias and ST/T wave changes. This is difficult at present because of the large electrical artefacts caused by blood flow and movement of leads in the high magnetic fields needed for spectroscopy.

In conclusion, phosphorus-31 magnetic resonance spectra were recorded from the normal myocardium during exercise during a new exercise protocol. There were no differences in high energy phosphorus metabolism during light steady state exercise, which suggests that the normal myocardium is adequately supplied by oxidative metabolism during exercise.

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